EFFECTS OF INSULIN TREATMENT ON EXPERIMENTAL DIABETES OF DOGS

By E. F. F. Copp *
(*From the Scripps Metabolic Clinic*)

Doctor John R. Williams, Rochester, New York, believes that "Dr. Copp's work is timely and extremely important. It affords experimental proof for the clinical observation of a number of workers. It should be extended to throw light on the problem of the source and regeneration of the islets.

An editorial councilor, in endorsing this discourse for acceptance by California and Western Medicine, says: "It appears to me a worthy presentation of an interesting aspect of diabetes, well presented and, I believe, well sponsored, as coming from the new Scripps Metabolic Clinic."

Doctor F. G. Banting, University of Toronto, closes his discussion of Doctor Copp's studies by saying: "The fact that Dr. Copp has been able to show the complete disappearance of hydropic degeneration of the beta cells of the islets of Langerhans in partially depancreatized dogs by insulin treatment is of great importance, because it provides experimental evidence which supports the clinical findings that, in order that there be a gain in tolerance, the islets of Langerhans must be relieved of all strain."

Doctor Frederick M. Allen, Morristown, New Jersey, states in his discussion that "the ability to produce specific island changes at will by dietary overstrain, and to arrest these changes by relieving the overstrain, is so significant that these simple experiments should be tried by those pathologists who still doubt the island theory of diabetes. The occurrence of this same degenerative process in experimental animals, and in human patients likewise, ranks among the important proofs of the identity of experimental and clinical diabetes."

Doctor Lovell Langstroth, San Francisco, considers that "Dr. Copp brings us experimental proof of the importance of maintaining a normal blood sugar in diabetic dogs. This fits in exactly with the clinical facts, and there is every reason to suppose that his results are directly applicable to humans."—Editor.

THE year 1889 probably marked the beginning of the study of experimental diabetes of animals. In that year, Mering and Minkowski [1] discovered, more or less by chance, that total pancreatectomy of dogs was followed by a severe and uncontrollable form of diabetes. This fact established a definite relationship between diabetes and the pancreas. Minkowski then tried, without success, to treat diabetes by feeding whole pancreas.

Until that time no great suspicion existed that the islands of the pancreas, described by and named after Langerhans in 1869, played any particular role in the metabolism of carbohydrates. Lewaschew [2] believed that the islands were simply modified acinar cells.

In 1884 Arnozan and Vaillard [3] noticed, after ligation of the pancreatic ducts in rabbits, that the acinar cells degenerated and were replaced by fibrous tissue. Schultze, in 1900, showed that after ligation of the ducts or in grafts of the gland the islands of Langerhans did not undergo destruction. Sscobolew,[4] in 1902, also noticed that there was atrophy of the insular tissue after ligation of the pancreatic ducts.

In 1901 Weichselbaum and Stangl [5] first observed and clearly described the so-called hydropic degeneration of the islands of Langerhans in human cases of diabetes.

In 1901 Opie [6] claimed that there was a direct relationship between the pancreatic islands and human diabetes, and noticed hydropic degeneration of the Beta cells of the islands.

In 1908 Zuelzer [7] prepared an alcoholic extract of the pancreas of recently fed animals which, on intravenous injection, was capable of greatly diminishing, in several animals, the glycosuria following pancreatectomy. The pancreatic veins of those animals from which the extract was prepared were ligated for an hour before killing them, in order to attempt to accumulate the hormone in the gland. Forschbach [8] confirmed Zuelzer's finding on diabetic dogs, but as the extracts contained a large amount of protein he considered the results were due to a febrile reaction following the injection of the antidiabetic hormone. So the use of their extract was abandoned.

MacCallum [9] ligated the ducts of the tail of the pancreas, and in a second operation several months later removed the rest of the pancreas and found that only a mild condition of diabetes followed. In a third operation, he removed the degenerated tail of the pancreas, and found that total diabetes resulted. This proved that the islands of Langerhans alone prevented the onset of diabetes, as the remnant removed was practically all islands. Unfortunately, an extract of this tissue was not made.

In 1913 Allen [10] first demonstrated experimentally on dogs that the production of diabetes was followed by typical pathological changes in the pancreas similar to those seen in the human. It was he who first noticed the hydropic degeneration of the Beta cells of the islands of Langerhans in dogs.

Knowlton and Starling [11] prepared extracts of the pancreas in weak acid solution, and found that a diabetic heart, when perfused outside the body, utilized less sugar than that of a normal heart similarly treated. Starling later believed that a mistake had been made somewhere, and abandoned further work along similar lines. Murlin [12] came very close to developing a potent pancreatic hormone, but made his extract alkaline instead of acid.

This long series of attempts to isolate the antidiabetic hormone was followed by the announcement of Banting's [13] discovery in 1921. He conceived the idea of preparing insulin in November, 1920, while working as assistant in physiology at the Western University at London, Canada, as a result of reading an article by Baron [14] published in Surgery, Gynecology and Obstetrics. Banting and Best ligated the pancreatic ducts of dogs for ten weeks, and then from the remnant, largely composed of island tissue, prepared a potent extract by thinly slicing and pounding up this tissue in chilled Ringer's solution.

By May, 1922, Best had so refined the extract that the first clinic to use insulin was started in the Christie Street Military Hospital, Toronto. Before that time, subcutaneous injections of insulin often

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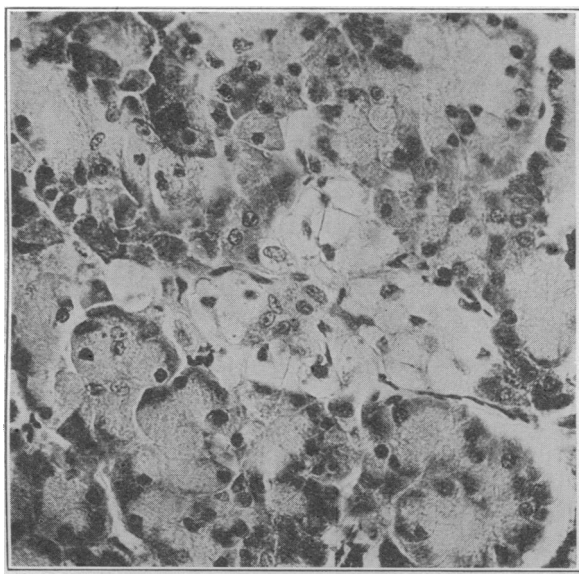

Figure 1

Dog. Advanced hydropic degeneration. Size of island evidently diminished by loss of cells. Contrast between vacuolated beta cells and surviving alpha cells under routine stain (eosin-methylene blue). The alpha cells appear in the center of the island.

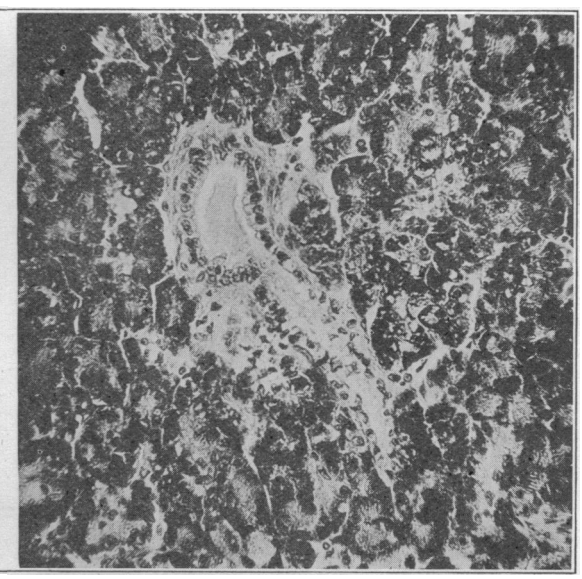

Figure 2

Dog. Advanced diabetes. A small duct opens into a large one. Some cells are vacuolated, others are not.

caused localized abscesses. Since that time very many papers have appeared regarding insulin treatment, and the general consensus of opinion at the present time is that insulin plus diet gives hope that even the severest case of diabetes may live comfortably and indefinitely.

The object of the present paper is to discover what changes are taking place in the pancreas, especially the islands, as a result of insulin treatment. Dogs, as a rule, were used in these experiments. The plan adopted was first to produce diabetes in dogs, as described by Allen.[15]

In this series of experiments some thirty animals were used, and dogs were found to be the best suited for this work. Cats, sheep, and goats did not stand the operations well, and it was almost impossible to produce diabetes in hogs, regardless of the amount of pancreatic tissue removed.

Each dog was submitted to several operations. At the first operation from eight-ninths to twelve-thirteenths of the total amount of pancreatic tissue was removed, and the small remnant of the gland was left around the major or minor duct. A specimen was saved for microscopic examination. The dog was then placed upon a high carbohydrate diet for approximately one month, during which time the animal was actively diabetic.

At the second operation, from twenty-six to thirty days later, a specimen of the gland was removed for microscopic examination. These sections showed that the alpha and gamma cells of the pancreatic islands remained in good condition, but that the beta cells showed the presence of so-called hydropic degeneration (Figure 1). The acinar cells remained perfectly normal. Vacuolation of some of the cells lining the ducts was also seen at times (Figure 2). At the close of the second operation, treatment with insulin was immediately started, and subcutaneous injections were usually made three to four times a day. These injections were as evenly distributed over the twenty-four hours as possible. Each dog was placed on a carefully weighed diet, usually being fed three times a day on lean meat.

A strictly normal level of blood sugar was aimed at and maintained during the whole length of this period. This program was kept up for from twenty-four hours to three weeks, and many specimens of pancreas from different animals were removed at varying intervals.

Allen [15] had previously tried to learn whether the hydropic degeneration of the beta cells of the islands was reversible or not, but was unable to arrive at any definite conclusion, because he stated "if pancreatic tissue is removed at the desired stage of maximal vacuolation, either the diabetes is already hopeless or the slight trauma of the operation makes it so, for it is impossible to stop the glycosuria thereafter, and with continued symptoms rapid destruction of islands occurs."

RESULTS

Complete disappearance of hydropic degeneration of the beta cells of the islands of Langerhans and the restoration of the islands to normal was found after a period of fourteen days of insulin treatment (Figures 3 and 4). About the ninth day vacuolation began to subside, but before this period very little recovery could be noticed.

Careless control of the diabetes showed that the functional overstrain on the islands had not been relieved, as they still showed hydropic degeneration. We have reason to believe that hyperglycemia, without sugar in the urine, will prevent the clearing up of the pathological picture of vacuolation of island cells.

Lack of careful control of the symptoms of diabetes in dogs is rapidly followed by acidosis. It is interesting to note that before insulin treatment of diabetes of dogs, it was almost impossible to produce a severe acidosis in these animals. Now it is com-

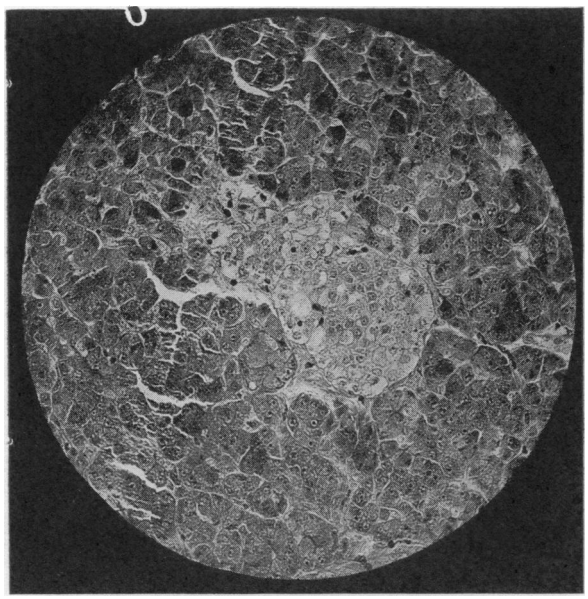

Figure 3
Dog 148. Pancreas showing hydropic degeneration of the beta cells after twenty-eight days of persistent glycosuria.

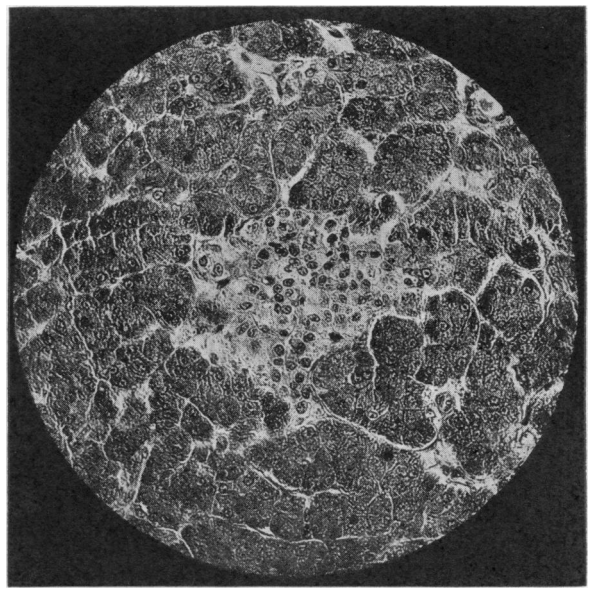

Figure 4
Dog 148 after fourteen days of insulin treatment, showing the restoration of beta cells and the return to normal of the island.

paratively easily done by suddenly stopping administration of insulin, but still keeping on with the same diet.

Severely diabetic dogs can be kept alive indefinitely by means of a careful diet plus insulin.

The glucose tolerance of diabetic dogs under insulin treatments will improve, often remarkably. We have been unable to definitely prove the formation of new islands, but vacuolation observed occasionally in centro-acinar cells and in the small ducts or cell cords seems to suggest an intimate relationship between these cells and the beta cells of the islands. We expect to later report on this relationship more definitely. The diabetic dog thrives best when strictly normal blood sugars are maintained at all times.

CO-RELATION TO THE HUMAN DIABETIC

The pathological changes of the pancreas as a result of diabetes, whether seen in the human or the dog, are almost identical. In both cases the beta cells of the islands become vacuolated as a result of functional overstrain. We have seen that the pathological changes of the pancreas of diabetic dogs return under careless control, and we believe the same occurs in the human.

It is, therefore, important to realize that only by careful control and by the maintenance of strictly normal blood sugars can we expect to have the already damaged organ in diabetes functioning at its best. Occasional presence of sugar in the urine means the probability that some of the beta cells of the islands are becoming vacuolated, and if repeated may mean the destruction of island tissue, with a further irreparable loss in food tolerance.

Just what is happening in the patient's pancreas when under good control is an interesting speculation, but when under poor control we most certainly know that specific pathological changes are taking place detrimental to the patient.

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DISCUSSION

F. G. BANTING, M. D. (University of Toronto, Canada)—In reading Dr. Copp's paper one feels regret that he has not reported upon this important work in greater detail. There is now little doubt that glucose directly stimulates the islets of Langerhans to produce insulin. The histological picture described in this paper provides evidence from the pathological standpoint. It has been found from a physiological standpoint that the secretion of insulin does not necessarily depend upon the nerve supply, since

when a portion of pancreas is transplanted under the skin and depleted of its nerve supply, there is no material alteration in the response to injected glucose. Furthermore, when the percentage of sugar in the blood is suddenly increased the islet cells are stimulated to produce an adequate amount of insulin to reduce the blood sugar to its normal level. From a clinical viewpoint, it has been found that, in cases of diabetes mellitus which have been maintained sugar-free and with a normal blood sugar, there is no decrease in tolerance, and in nearly all cases, particularly in children, there has been a considerable increase in tolerance. On the other hand, in cases which have continually shown glycosuria there is no gain in tolerance, and usually there is lowering of tolerance.

The fact that Dr. Copp has been able to show the complete disappearance of hydropic degeneration of the beta cells of the islets of Langerhans in partially depancreatized dogs by insulin treatment is of great importance, because it provides experimental evidence which supports the clinical findings that, in order that there be a gain in tolerance, the islets of Langerhans must be relieved of all strain.

FREDERICK M. ALLEN, M. D. (Physiatric Institute, Morristown, New Jersey)—The importance of the study of pancreatic pathology cannot be overestimated. The hydropic degeneration of island cells can be easily and clearly demonstrated. It is biologically unique as an example of anatomic degeneration, due to overstrain of an endocrine function. It is one of the strongest links in the proof that the anti-diabetic function of the pancreas actually resides in the islands. Experiments like those of MacCallum are not decisive, because the ligated pancreas remnant of the dog never consists exclusively of island tissue. A strong support is furnished by Macleod's observations in fish, that insulin is obtainable from the portions of the pancreas which contain islands, and not from the portions containing no islands. But the ability to produce specific island changes at will by dietary overstrain, and to arrest these changes by relieving the overstrain, is so significant that these simple experiments should be tried by those pathologists who still doubt the island theory of diabetes.

The occurrence of this same degenerative process in experimental animals and in human patients likewise ranks among the important proofs of the identity of experimental and clinical diabetes. It has several fundamental therapeutic applications. It furnishes the anatomical explanation of the progressiveness of diabetes. One of the traditional errors that was hardest to overthrow was the belief in the inherent progressiveness of diabetes. There is now agreement of experimental and clinical proof that diabetes is progressive when the island function is overtaxed, and is not progressive when that function is spared. The means of overtaxing the function are also significant. Not only carbohydrate, but also the total calories and body weight influence the island function in both dogs and patients. It is unfortunate that it should still be necessary to repeat this very simple and easily demonstrated fact. Increased calories in the form of fat increase the tendency to glycosuria and acidosis, and increase the insulin requirement in both patients and animals. The pernicious fad of high fat diets rests only upon inconclusive observations that some patients can be freed from glycosuria and acidosis, in spite of such diets. Accurate comparisons of low and high fat diets have never failed to confirm this fact, which, besides its practical importance, has a deep theoretical significance with reference to the role of insulin in the bodily chemistry.

LOVELL LANGSTROTH, M. D. (490 Post Street, San Francisco)—Dr. Copp brings us experimental proof of the importance of maintaining a normal blood sugar in diabetic dogs. Diets which throw a functional overstrain on the islands lower the tolerance of the animal apparently by causing degeneration of the beta cells. Diets which maintain a normal blood-sugar level often result in a marked gain in tolerance, and this we now know is associated with disappearance of the degenerative changes. This fits in exactly with the clinical facts, and there is every reason to suppose that his results are directly applicable to humans.

In clinical diabetes we see different responses to the same treatment. In two properly treated cases of the same severity one will show a marked increase in tolerance; the other none. This appears to be due to some inherent difference in the cells of the two individuals, to an ability in one to "come back" under rest, in other words. This difference in regenerative power brings up some interesting problems. Is this the same tissue quality which makes one individual so resistant to diabetes that his tolerance falls very slowly over the course of years, while another offers no barrier to its progress and soon becomes a total diabetic? If we may speak of diabetes as a degenerative disease, is the resistance to this degeneration largely a question of inheritance as the occurrence of severe infantile diabetes would certainly make it appear? And if we inherit poor resistance to such degeneration, what are the factors in the lives of parents which withhold this essential quality from the offspring? In this connection the new experimental work on food and sunlight in animals comes to mind at once. One wonders whether the second or third generation of rats fed on suboptimal diets and showing diminution in size, vigor, and fertility, might not be made to show some such marked loss of resistance to degeneration as is evidenced in a diabetic infant.

These problems are at the root of our understanding not only of diabetes, but of all degenerative diseases and such interesting experimental work, as this gives us hope of their solution in the near future.

JOHN R. WILLIAMS, M. D. (388 Monroe Avenue, Rochester, New York)—That insulin may have some regenerative or restorative action on the pancreas has been the hope of every worker in diabetes. Clinical evidence is slowly accumulating which suggests that such action is taking place. I have a series of both children and adults who were carefully controlled and whose limitations were established before the advent of insulin. The increase of pancreatic efficiency is more evident in adults than in children, although general clinical improvement is striking in both groups.

The value of maintaining the body chemistry as nearly normal as possible in diabetes can scarcely be questioned. The difficulty of so doing in severe cases is another matter. If it has to be done at the cost of marked undernutrition, it is an open question. The cases in my series who are permitted to maintain an approximately normal nutrition not only feel better, but appear clinically improved, and show no evidence of breaking down. Some of these cases have carried high blood sugars and urine sugar, and in other ways have violated the conventional beliefs as to the importance of diabetic standards. It should always be borne in mind that insulin activity in the body and glucose metabolism rarely synchronize; that the severe and uncontrolled diabetic under insulin therapy during the day may have periods of hypoglycemia as well as hyperglycemia. These alternating states may not be without benefit to the severe patient, particularly if a good state of nutrition is maintained. In some of my severe cases there is evidence to support this possibility.

My clinical experience is not in accord with that of Dr. Allen, if I understand him correctly. Without insulin, on the plan of undernutrition alone, my cases were greatly benefited for a considerable period of time; eventually they failed, and although the expectancy of life was extended as much as 100 per cent, the disease progressed and the patient finally succumbed either to inanition or the diabetes. In have noticed no such regression since the use of insulin.

Dr. Copp's work is timely and extremely important. It affords experimental proof for the clinical observation of a number of workers. It should be extended to throw light on the problem of the source and regeneration of the islets. The very great desirability of careful autopsy study of all insulin-treated diabetics who may succumb either to diabetes or other causes must be evident in this connection. The Scripps Metabolic Clinic is to be congratulated on the character of its research.

What a paradox that the very man to whom the world looks for advice as to proper food, rest and exercise, must eat cold grub grabbed on the wing, sleep hanging on a hook by the telephone, and depend for his exercise on cranking the flivver.—Medical Pocket Quarterly.